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EDITORIAL REVIEW

Pathophysiology of low renin syndromes: Sites of renal renin secretory impairment and prorenin overexpression

The renin-angiotensin-aldosterone system (RAAS) plays an important role in cardiovascular and electrolyte regulation in health and disease [1–3]. Although peripherally produced renin plays a role, renin secretion (RS) from juxtaglomerular (JG) cells initiates the endocrine cascade by cleaving angiotensin I from a precursor molecule which in turn generates angiotensin II (Ang II) to complete the physiologic cycle by stimulating vascular smooth muscle contraction and aldosterone secretion and by inhibiting RS, probably through an exaggerated negative feedback hyporesponsivity involving Ca. Renal RS is modulated by intracellular Ca (Ca_i), K, Cl, Na and pH, whereas Ang II and aldosterone modulate Ca_i, K, Cl, Na and pH in their mechanism of action. Thus, RAAS is activated by and subsequently modulates Ca and chemiosmotic constituents (ions and water driven by proton movement). The specific sites and mechanisms of action of Ca and chemiosmotic forces (K, Cl, pH and water movement) were considered in some detail in a previous review, with only a brief consideration of the intrarenal control of renin and prorenin secretion, renin profile, and mechanisms of subcellular dysregulation in several specific cases of renin disorder [4].

Most renin disorders present a biphasic plasma renin profile. The pathogenesis of benign renovascular hypertension during normal salt balance is most accurately described by an initial elevation of plasma renin activity (PRA), aldosterone, plasma volume, and retention of salt and water immediately after renal artery constriction [1]. This initial phase is characterized by secretory hyperresponsivity since an identical stimulus provokes an exaggerated response [5]. Later on PRA returns to "normal" or below normal despite maintained stimulation. This latter phase is termed renin hyporesponsive because the PRA is much lower than expected for an identical level of stimulation (Fig. 1A). The pathogenesis of experimental congestive heart failure also demonstrates a biphasic PRA profile [6]. Constriction of the thoracic inferior vena cava initially leads to an increase in renin and aldosterone secretion, sodium retention, and plasma volume expansion. Later on during maintained constriction PRA returns to normal or below. Again, the initial phase was demonstrated to be renin hyperresponsive, whereas the later phase was renin hyporesponsive (Fig. 1B). Diabetic induction with streptozotocin is a final example of the biphasic PRA profile often seen in the pathogenesis of renin disorders [7]. One week after diabetic induction PRA increases almost twofold (Fig. 1C). However, by the third and fourth week PRA reached subnormal levels. Renin hyporesponsivity has been observed in a number of other clinical and experimental renin disorders [4].

Possible mechanisms of renin hyporesponsivity will be the chief focus of this brief review. First, we will briefly describe the phenomenon in diabetes mellitus and other clinical disorders classified along the lines of renin hyporesponsivity [4]. Second, we will critically evaluate what is currently known about intrarenal control of renin and prorenin secretion and the role of physical equilibrium of the afferent arteriole in modulating secretion. The role of the macula densa in the processing of prorenin to renin will be reviewed critically in view of a recent line of direct evidence as well as recent proposals for a physiological as well as pathophysiological role of prorenin itself. Using allometric analysis it has been observed that kidneys from animals of greater body weight secrete a greater proportion of their total secretory package as prorenin. The significance of this observation will be discussed with reference to allometric analysis of other aspects of renal function. Third, we will identify subcellular sites of potential dysregulation in renin secretory hyporesponsivity, and therefore set the stage for further analysis of the renin secretory pathway at the biochemical and molecular levels in an attempt to find successful therapeutic strategies.

Diabetes mellitus and low renin syndrome

Stages of renin hyporesponsiveness characterize a fair number of clinical disorders (Table 1). As shown in Figure 1, the renin-hyporesponsive phase is usually associated with established disease in hypertension, heart disease, and diabetes. Long standing diabetes (17 years) was reported with over 70% as "low-renin" [14]. Primary aldosteronism is a typical example of the blunted renin responsiveness [8]. The hyporesponsivity remains the same whether the cause of aldosterone excess is aldosterone-producing adenoma, idiopathic hyperaldosteronism, intermediate hyperaldosteronism, or glucocorticoid-remediated hyperaldosteronism [4]. Thyroid deficiency is another example [9]. The majority of acromegalic patients have a generalized lower PRA compared to normals, and hypertensive acromegalics have an even more profound hyporesponsivity to conventional stimulation [10]. Pituitary deficiency is an established case of renin secretory hyporesponsivity [11–13]. Additional cases include Blacks (particularly African Americans) [16], late stages of experimental pheochromocytoma [17], and chronic salt excess [18]. Including the recently described New's syndrome of apparent mineralocorticoid excess and Gordon's syndrome of hypertension with hyperkalemia despite normal GFR, renin secretory hyporesponsivity has been identified in over 23 disorders [4], including polycystic kidney disease and most cases of end-stage renal disease. It is this whole matrix of

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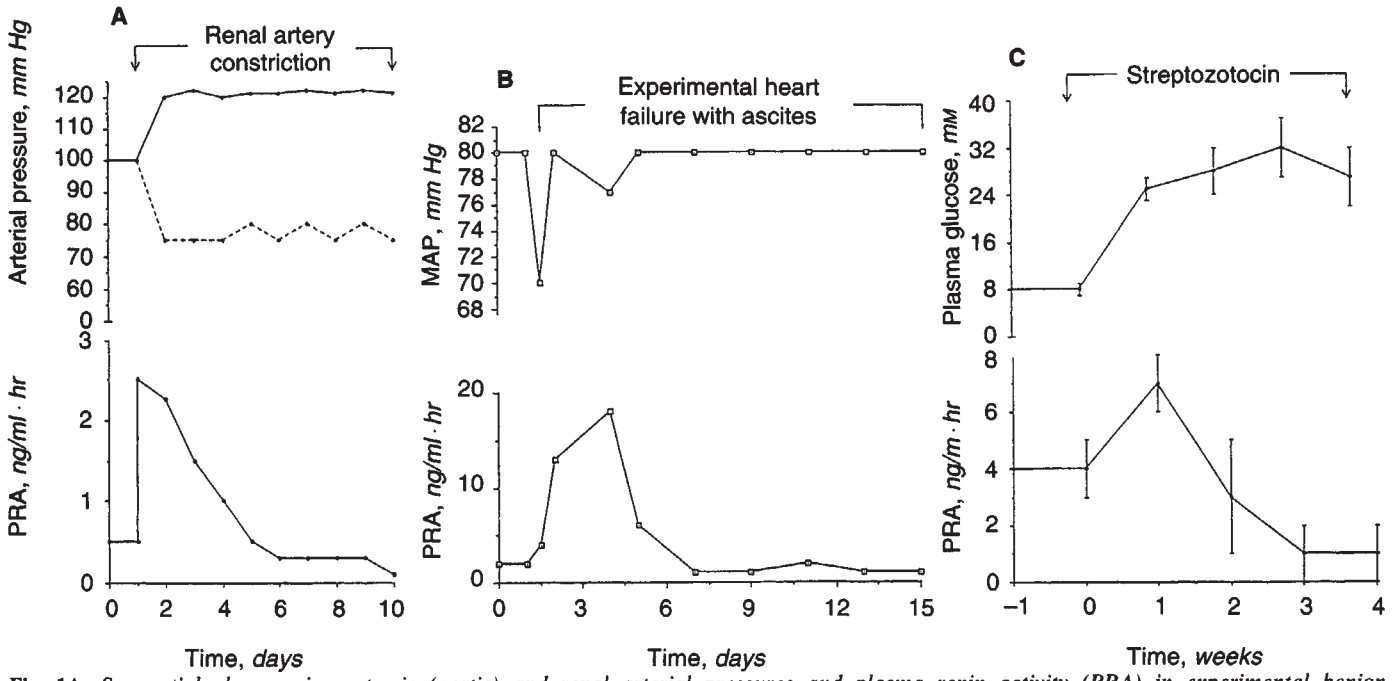

Fig. 1A. *Sequential changes in systemic (aortic) and renal arterial pressures and plasma renin activity (PRA) in experimental benign hypertension.* Used with permission from Dzau et al [175]. **B.** Responses in the conscious dog to thoracic inferior vena caval constriction and the development of moderate congestive heart failure. Used with permission from Watkins et al [6]. **C.** Plasma levels of glucose and renin activity in streptozotocin-diabetic rats as a function of duration. Used with permission from Kikkawa et al [7].

Table 1. Basal plasma renin activity (PRA), renin secretion and secretory responsiveness in extreme cases of low renin syndrome

Case	Basal PRA or renin secretion	Stimulated PRA or renin secretion	Reference
Diabetes mellitus (type I)	low (normal)	blunted	[14]
Diabetes mellitus (type I)	low (normal)	blunted	[15]
Primary aldosteronism	low	blunted	[8]
Thyroid deficiency	low	blunted	[9]
Acromegaly	low	blunted	[10]
Pituitary deficiency	low	blunted	[11]
Black Americans	low	blunted	[16]
Pheochromocytoma	low	blunted	[17]
Chronic salt excess	low	blunted	[18]

Diabetes mellitus, acromegaly, and pheochromocytoma may also have high or "normal" basal PRA or renin secretion, but in established phases of these diseases the predominant feature is low renin.

clinical (and experimental) disorders characterized specifically by renin secretory hyporesponsivity that has been termed low renin syndrome (LRS) [4].

There are many reasons why these seemingly "different" clinical and experimental disorders should not be clustered under a single syndrome, except one. LRS would constitute an immediate source of readily available information pointing to similarities, rather than differences. In several cases of LRS such as experimental diabetes mellitus [19], mineralocorticoid excess [20], pituitary deficiency [13, 21], Blacks in general [22], and renal insufficiency [23], detailed information is already available, even at the molecular level. In all cases examined there is one striking similarity: renin secretory hyporesponsivity [4]. In the context of this similarity and a brief consideration of what is currently understood about the renin secretory pathways at the subcellular level, it has been suggested that from signal recognition, transduction, and transformation to production from rough endoplasmic reticulum (ER) to Golgi stacks and finally to secretion-ready mature secretory granules releasing their contents to the extracellular space there are at least 23 separate molecules that are potential targets of attack in any of the disorders clustered under LRS [24]. To unify the seemingly disparate renin (and prorenin) profiles demonstrated in LRS, one recent review approached the problem from the point of view of synthesis, processing and export at the intracellular level while backing into problems encountered during renin abnormal expression [4]. The role of Ca and chemiosmotic forces was considered in some detail [4]. That approach led the way to the discovery of LRS as a pervasive clinical disorder characterized chiefly as a disease of defects in intracellular processing, trafficking, and exporting [4]. In addition, the approach pointed to more than few significant intracellular regulatory sites which may be prime targets for pathological attack, and therefore therapeutic focus [4]. In opposition to the earlier approach, however, here we shall attempt to see in what ways a fair number of the seemingly different disorders which constitute LRS may lead to a similar picture, and thereby provide the most cogent scientific justification for classifying them as LRS. Although much is now known in terms of plasma renin abnormal expression in LRS cases such as rheumatoid vasculitis [25], Parkinson's disease, amyloidosis and progressive autonomic failure [26], it is from diabetes mellitus that a great deal of relevant information has arisen [19], particularly

low-renin diabetic hypertension [22] where the pathophysiology is well described.

Several pathologies have been documented to be associated with LRS. In the late stages of renovascular hypertension (phase III, characterized by renin hyporesponsivity [1]) we often see atherosclerosis, arterial fibrodysplasia, renal artery thrombosis (or aneurysms), polyarteritis nordosa, neurofibromatosis, along with a variety of abnormal electrolyte imbalances [27]. In low-renin essential hypertension nephrosclerosis is sometimes a predominant renal feature. It has been postulated that the vascular changes of nephrosclerosis cause a reduction in the compliance of the afferent arteriole and that this underlies the mechanism of renin hyporesponsivity [28]. This hypothesis will be considered below in the general context of the site(s) of impairment. In New's syndrome of apparent mineralocorticoid excess (particularly in adolescents) we see a profound hypokalemia, suppressed ACTH and all known corticosteroids, and refractoriness to spironolactone treatment [29]. The clinical features of Gordon's syndrome is often muscle weakness, shortness of stature, intellectual impairment, and excess dental abnormalities [30]. In hyporeninemic hypoaldosteronism we see nonoliguric renal disease, hyperkalemia and hyperchloremic acidosis as the chief features defining LRS [31]. In acromegaly the profile often resembles that reported in low-renin essential hypertension and primary aldosteronism [32]. In diabetes mellitus diffuse glomerular lesions and the so-called Kimmelstiel and Wilson lesions of the microvasculature have been reported [3]. The former are most frequently associated with diabetic nephropathy (a classic case of LRS) with the associated proteinuria and eventual renal failure [3]. Christlieb [3] pointed out that arteriosclerotic changes are seen in LRS cases beside diabetics, but what is striking in diabetes is that the arteriolar hyalinization attacks both the afferent and efferent arterioles, and in extreme cases it closes the lumen of the afferent. These changes have been found to have profound effect on RS [33]. Perhaps equally significant is the observation of a strong correlation between the severity of the glomerular nodular lesion and renin responsivity factors [3]. At the macroscopic level renin hyporesponsivity in diabetes is often associated with nephropathy, neuropathy, and ketoacidosis [34].

Intrarenal control of renin and prorenin secretion

The JG cell occupies a unique position among secretory cells in terms of the variety and complexity of its control mechanisms. Its position in the wall of the afferent arteriole allows it to respond to some biophysical manifestations of blood pressure as well as to biochemical effects of hormones and neurohormones in plasma. Additionally, the JG cell is subject to sympathetic control and is also influenced by aspects of tubular function by way of the macula densa. All these diverse signals impinge on the JG cell, which must somehow integrate them to provide the appropriate measure of RS and, therefore, RAAS activation. An attempt to describe briefly these routes and control mechanisms is given below. The next two sections attempt to describe how the integration of the various control mechanisms might be achieved. Central to the consideration of integration are three features: two related to the environment of the JG cell and one to its secretory activity. First, any myogenic activity of the afferent arteriole in support of renal blood flow autoregulation will influence the "hemodynamic environment" of the JG cell and its secretory function. Secondly, the requirement for the afferent arteriole to be in physical equilibrium provides a mechanism for defining the hemodynamic environment of the JG cell and its secretion of renin. Thirdly, the differential release of renin and prorenin from the kidney is a macula densa-mediated phenomenon which ensures a particular degree of activation of RASS by the afferent arm (afferent arteriole) is matched to the characteristics of the efferent arm (the nephron). The implication of the latter point is that the importance of circulating renal prorenin resides not in its potential to contribute to the expression of RAAS via activation, but in its very inactivity.

Intrarenal control of renin secretion

Classically, the renin secretory activity of JG cells is controlled by a number of discrete sensors which differ in stimulus modalities and pathways for signal transduction. These routes have been described as baroreceptor, neurogenic, hormonal, and macula densa regulation [35–38].

Baroreceptor control. There is ample evidence of an inhibitory effect of renal perfusion pressure on RS mediated at the level of the afferent arteriole [39, 40]. The original concept of a baroreceptor responding to pressure [41] has been expanded to include the afferent arteriolar radius and tissue pressure contributions to transmural wall tension [35] and its more detailed variant, stretch [42]. More recent studies [43] suggest that changes in shear stress related to changes in pressure-induced flow might also contribute to the renin response to pressure. Although the proper functioning of the baroreceptor in situ requires the afferent arteriole to be intact, direct evidence suggests that mechanical deformation can alter renin release at the level of the individual JG cell [44]. Calcium plays a vital role in coupling mechanical stimulation of the JG cell to inhibition of RS. Increased stretch increases Ca influx into JG cells which inhibits RS, with the increased Ca-conductance mediated by stretch-activated channels (SACs) [42, 44]. The Ca-dependence of baroreceptor function is demonstrated by the observations that Ca-channel blockers prevent the inhibitory effect of high pressure on RS, and the inhibitory effect of high pressure on RS depends on extracellular Ca [38]. Recently, it has been suggested that Ca_i exerts its inhibitory effect on RS via activation of KCl and water efflux from the cell mediated by a Ca-activated chloride channel in the surface membrane [45]; and via deactivation of KCl and water influx into secretory granules mediated by a KCl-H exchange transporter in the granular membrane [4]. Although the mechanism for signal transduction at the JG cell membrane remains to be fully defined, blood pressure effectively remains the "physiological variable" to which the baroreceptor responds [46].

Neurogenic control. Since the description of the innervation of the JG apparatus [47], an impressive amount of evidence has accumulated suggesting that RS is stimulated by a β-adrenergic mechanism [48]. β-Adrenergic receptors are coupled to adenylate cyclase in many tissues [49] and a cAMP-mediated effect for RS is therefore implicated. Compounds such as glucagon and forskolin, which are known to increase cAMP, stimulate RS [50, 51]. Although β-adrenergic agonists can stimulate RS in the absence of a fully functional baroreceptor mechanism [52], *in situ* it is likely that hemodynamic effects of sympathetic activity can interact to modify RS. Thus, RS is affected by low

frequency renal nerve stimulation, sodium excretion by medium, and vascular resistance by high frequency [53].

Hormonal control. A variety of locally-produced and circulating compounds can affect RS. These include angiotensin, vasopressin, ANP, parathyroid hormone, glucagon, endothelin, prostaglandins, histamine, and dopamine [37, 48]. Perhaps the most important of these in terms of the feedback control of RAAS is angiotensin II. The inhibitory effect of angiotensin II on RS requires extracellular Ca [54] and is attenuated by Ca-entry blockade [55], suggesting the inhibitory effect is mediated by calcium influx [55].

Macula densa control. The close apposition of the macula densa with the renin-secreting JG cells provides the anatomic basis for the direct control of RS by nephron function. The original hypothesis of Vander [56] proposed an inverse relationship between RS from JG cells and sodium load at the macula densa. The demonstration of an inverse relationship between sodium chloride delivery to the macula densa and RS in microdissected, perfused JG apparatus [57] has provided convincing support for the original hypothesis. Ion substitution experiments in rats, however, indicate the importance of chloride rather than sodium in mediating the macula densa control of RS [58, 59]. These observations have been strengthened by experiments in which the substitution of chloride with gluconate suppress RS from superfused rat glomeruli [43]. Moreover, the suppression of RS by chloride substitution was reversed by removal of Ca from the superfusate, further demonstrating the central role played by Ca in the control of RS, even when the effector is via a macula densa pathway.

Role of physical equilibrium of the afferent arteriole in determining renin secretion

The afferent arteriole is intimately involved in two important renal phenomena. It is the primary site for the vascular resistance changes that mediate RBF autoregulation and it contains the modified smooth muscle cells, the JG cells, that release renin. The interdigitation of the smooth muscle responsible for blood flow autoregulation and the JG cells responsible for RS suggests a close functional relationship. Thus, it has been suggested that RS mediates autoregulation [60–62] and also that autoregulatory responses determine RS [63, 64]. More specifically, since there is ample evidence that the afferent arteriole acts as an autonomous baro- or stretch receptor controlling RS [39, 40], and because the afferent arteriole demonstrates a myogenic response to pressure [65, 66], it is necessary that any realistic consideration of RS from JG cells *in situ* must take into account the concurrent myogenic activity of the smooth muscle cells (this is of particular importance for the kidney as its vascular response to changes in perfusion pressure are large, leading to near-constant RBF over a wide range of blood pressures). Although opinion is divided, a fair amount of evidence suggests that afferent arteriolar smooth muscle is unresponsive to angiotensin [65, 67] and that the primary intrarenal site for its vasoconstrictor activity is the efferent arteriole [68]. Therefore, the direct contribution of RS to afferent arteriolar contractile activity is likely to be small. Rather, it is the myogenic activity of the afferent arteriole which will set the hemodynamic environment of the arteriole and which may, in consequence, determine RS [64]. Similarly, humoral agents and neurotransmitters may well affect the JG cell directly, but *in situ* any concurrent action on vascular smooth muscle activity is likely further to influence RS by altering the hemodynamic environment of the afferent arteriole [53].

The myogenic response of the vasculature to increased pressure was first proposed almost a hundred years ago by Bayliss [69], but only recently has enough evidence provided to define the subtle and elusive mechanism. Central to any description of the myogenic response is the control loop initiating and maintaining contractile activity within the arteriolar wall. Although it has been suggested that myogenic activity adjusts itself to keep wall tension constant [70], such a control mechanism may be unnecessarily complicated since the most basic "feedback loop" operating within a blood vessel is the vessel's maintenance of physical equilibrium and its response to physical dysequilibrium. The implications for blood vessels of their requirement for physical equilibrium was first explored by Burton [71] and later incorporated into mathematical descriptions of the myogenic response and RBF autoregulation [70, 72, 73]. Most recently, a mathematical model for RBF autoregulation based on equilibrium theory [74] has been extended to include the prediction of RS [63]. The salient features of the model are illustrated in Figure 2 and are summarized as follows:

(1) Blood flow through the afferent arteriole is determined by the perfusion pressure and the arteriolar radius.
(2) The arteriolar radius is determined by the balance of two opposing forces: a distending force and a constricting force. Under stable conditions, the radius of the blood vessel will be such that these forces are equal and opposite (that is, the vessel is in physical equilibrium). Changing these conditions (such as changing blood pressure) will change the forces and bring the vessel into physical dysequilibrium; the radius will change accordingly to reestablish equilibrium (that is, if the change in blood pressure is such that distending force > constricting force, then dilation will occur; if distending force < constricting force, then constriction will occur).
(3) The distending force is related to the LaPlace equation, and is dependent on internal and external arteriolar radii, blood pressure, and tissue pressure.
(4) The constricting force is made up of two components: a passive tension related to the elastic properties of the vessel and the vessel radius, and an active tension which comprises the myogenic response of vascular smooth muscle to stretch [42]. Stretch (which is determined by hemodynamic factors such as blood pressure and vessel radius) activates SACs in cell membranes to increase Ca influx and so increase tension development.
(5) JG cells, being modified smooth muscle cells, also respond to stretch with a modified Ca influx. RS is then inversely related to Ca_i [4].

Figure 3A is an equilibrium diagram illustrating the way in which the requirement for physical equilibrium determines arteriolar radius for a given set of hemodynamic conditions [71]. The dashed line shows how the calculated distending force varies with arteriolar radius at a given blood pressure (100 mm Hg). The solid line shows how the calculated constricting force (active plus passive tensions) varies with arteriolar radius at the same pressure. The radius at which the lines intersect is stable

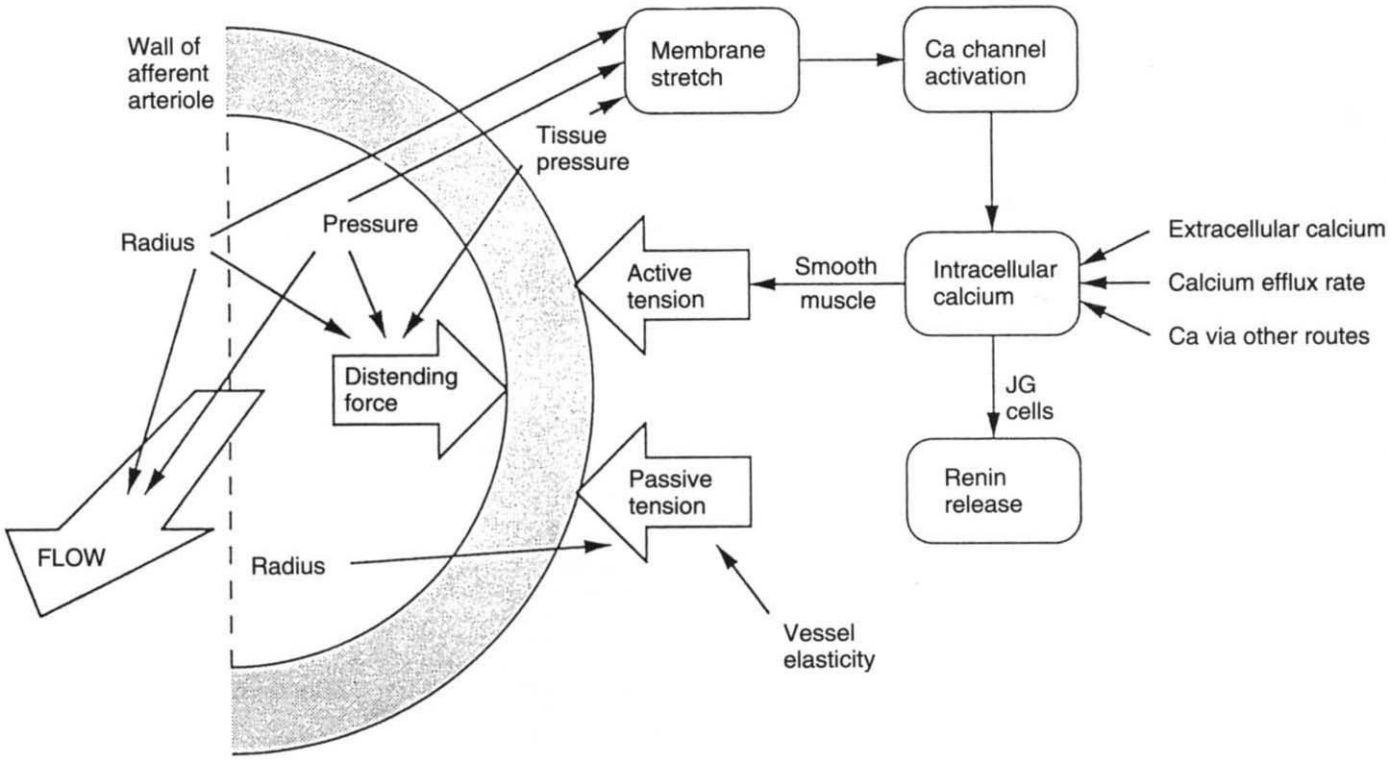

Fig. 2. *Features of the afferent arteriole in its regulation of renal blood flow and renin secretion.* Key features are that: (1) blood flow through the afferent arteriole is determined by the perfusion pressure and the arteriolar radius. (2) The arteriolar radius is determined by the balance of two opposing forces—a distending force and a constricting force. Under stable conditions, the radius of the blood vessel will be such that these forces are equal and opposite (that is, the vessel is in physical equilibrium). Changing these conditions (that is, changing blood pressure) will change the forces and bring the vessel into physical dysequilibrium; the radius will change accordingly to reestablish equilibrium (if the change in blood pressure is such that distending force > constricting force, then dilation will occur; if distending force < constricting force, then constriction will occur). (3) The distending force is related to the LaPlace equation, and is dependent on internal and external arteriolar radii, blood pressure, and tissue pressure. (4) The constricting force is made up of two components: a passive tension related to the elastic properties of the vessel and the vessel radius, and an active tension which comprises the myogenic response of vascular smooth muscle to stretch. Stretch (which is determined by hemodynamic factors such as blood pressure and vessel radius) activates SACs in cell membranes to increase Ca influx and so increase tension development. (5) JG cells, being modified smooth muscle cells, also respond to stretch with a modified Ca influx. Renin secretion is then inversely related to Ca_i. Adapted from [74].

since distending force equals constricting force. Radii to the left and right of that point are unstable as dysequilibrium prevails. To the left, distending force is greater than constricting force so the vessel dilates and the radius moves towards the stable radius. To the right, constricting force is greater than distending force so the vessel constricts and the radius moves towards the stable radius. Note that as the radius moves, the magnitudes of the tensions vary (for example, although movement towards the stable radius from a larger radius involves a reduction in the myogenic response, the constricting force is still greater than the distending force so the radius continues to decrease) illustrating the iterative, feedback nature of operation. Thus, a change in radius changes the myogenic response which changes the radius, which changes the response, etc. [63, 74].

In practice, however, it is not a change in radius that initiates dysequilibrium. It is a change in some other factor which alters the distending/constricting forces and so causes the radius to shift to a new stable value. For example, a change in blood pressure produces an autoregulatory adjustment in renal resistance (that is, afferent arteriolar radius). This is illustrated in Figure 3B, which shows the distending and constricting curves for two different pressures. The stable radius at each pressure occurs at the intersection of the corresponding distending and constricting curves. At 100 mm Hg, the stable radius is 9.78 μ. When the pressure is raised to 140 mm Hg, the altered dependencies of distending and constricting forces on radius is such that the stable radius is now 9.10 μ. On increasing the pressure from 100 to 140 mm Hg, according to the requirement for equilibrium illustrated in Figure 3B, the vessel must adjust its radius to this new value. A rise in blood pressure is therefore met with a reduction in radius, a rise in resistance, and blood flow autoregulation [63, 74].

The "search" for a new stable radius that characterizes the response to dysequilibrium involves a dynamic interplay between changing radius and changing distending, passive, and active (myogenic) tensions. Once the new radius is reached, however, the smooth muscle cells will demonstrate a level of active tension development determined by their Ca_i, which in turn will be determined by the degree of "stretch". Similarly, the JG cells (being modified smooth muscle cells sharing a similar afferent arteriolar environment) will also demonstrate a similar intracellular Ca. Since RS is Ca-dependent and is inversely related to intracellular Ca [4], RS can be calculated for any set of hemodynamic parameters once the afferent arteriole

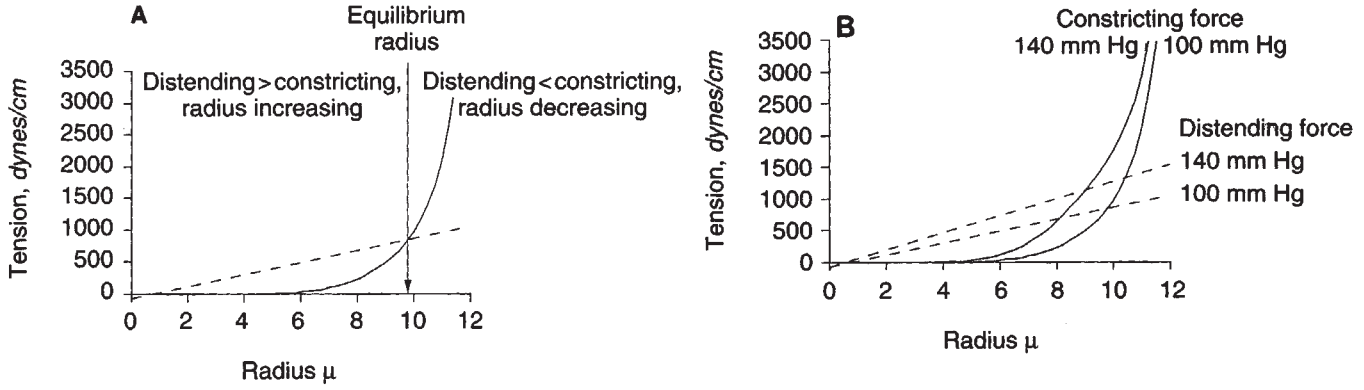

Fig. 3. *Equilibrium diagrams showing the effects of some consequences of dysequilibrium.* **A.** The way in which the requirement for physical equilibrium determines arteriolar radius for a given set of hemodynamic conditions. The dashed line shows how the calculated distending force varies with arteriolar radius at a given blood pressure (100 mm Hg). The solid line shows how the calculated constricting force (active plus passive tensions) varies with arteriolar radius at the same pressure. The radius at which the lines intersect is stable since distending force equals constricting force. Radii to the left and right of that point are unstable as disequilibrium prevails. To the left, distending force is greater than constricting force so the vessel dilates and the radius moves towards the stable radius. To the right, constricting force is greater than distending force so the vessel constricts and the radius moves towards the stable radius. Note that as the radius moves, the magnitudes of the tensions vary (such as, although movement towards the stable radius from a larger radius involves a reduction in the myogenic response, the contricting force is still greater than the distending force so the radius continues to decrease) illustrating the iterative, feedback nature of operation. Thus, a change in radius changes the myogenic response which changes the radius, which changes the response, etc. **B.** The distending and constricting curves for two different pressures. The stable radius at each pressure occurs at the intersection of the corresponding distending and constricting curves. At 100 mm Hg, the stable radius is 9.78 μ. When the pressure is raised to 140 mm Hg, the altered dependencies of distending and constricting forces on radius is such that the stable radius is now 9.10 μ. On increasing the pressure from 100 to 140 mm Hg, according to the requirement for equilibrium, the vessel must adjust its radius to this new value. A rise in blood pressure is therefore met with a reduction in radius, a rise in resistance, and blood flow autoregulation. Adapted from [74].

achieves equilibrium. It should be noted that, within the above model, Ca_i and therefore RS is a consequence of the way the afferent arteriole responds to its requirement for equilibrium. In other words, the myogenic response to hemodynamic variables such as pressure sets the conditions for RS.

The above model fairly successfully predicts RBF and RS under a variety of experimental conditions [63, 74]. These are summarized below:

(1) Increased RS in the sub-autoregulatory range and its suppression over the autoregulatory range of renal perfusion pressures.
(2) Increased RBF and RS with increased tissue pressure.
(3) Changes in the pressure dependence of RBF and RS with changes in extracellular Ca.
(4) Increased RS and RBF with increased Ca efflux.
(5) Effects of vasoconstrictors on RBF and RS depends on Ca and equilibrium status.

Generally, both experimentally and within the model, maneuvers which tend to produce vasodilation and increase flow tend to increase RS. However, some studies have indicated that this direct relationship between vascular resistance/flow and RS can be reversed [51, 75]. Given, within the above model, the similar determination of JG cell and smooth muscle Ca_i, the exposure of both cell types to similar hemodynamic environments, and the common dependence on Ca_i for RS and active tension generation, any uncoupling of flow from RS within the model would be a surprising and important prediction.

Figure 4 shows one qualitative comparison of model predictions with experimentally derived data from isolated perfused rat kidney. Using the perfused kidney, a recent report demonstrated that forskolin (an activator of adenyl cyclase) increased RS with a small increase in flow, and that simultaneous high K-depolarization reversed the renin secretory and flow effects of forskolin [51]. On the other hand, although the enhancement of RS by forskolin was reversed by increased perfusion pressure, flow was further increased rather than decreased by this maneuver. On its own, high K-depolarization suppressed both RS and flow. Evidence suggests that cAMP stimulates RS by increasing Ca efflux [38], so the effect of forskolin was simulated in the model by an increase in the Ca efflux rate coefficient of JG and smooth muscle cells (Fig. 2). High extracellular K can increase Ca-influx by depolarizing cell membranes [38], so this maneuver was simulated by increasing the activation of membrane Ca channels (Fig. 2). Although differing in the absolute magnitude of the percent changes from control values, the directions in which theoretical flow and RS move in response to the various simulations of experimental maneuvers are consistent with the actual data (Fig. 4). In particular, high K blunts the forskolin-induced increases in both RS and flow, but the inhibitory effect of high pressure on forskolin-induced RS is accompanied by the experimentally observed enhancement of flow.

These divergent effects of high K and increased pressure on forskolin-induced changes in RS and flow may be explained within the context of the model. The effect of increased pressure is mediated by stretch-induced changes in Ca influx; however, stretch is determined by factors other than perfusion pressure, most notably arteriolar radius. On the other hand, the depolarizing effect of high K on the cell membrane is direct and effectively independent of hemodynamic parameters such as pressure and radius. Thus, the increase in Ca_i caused by high K will increase the constricting force within the model, but this

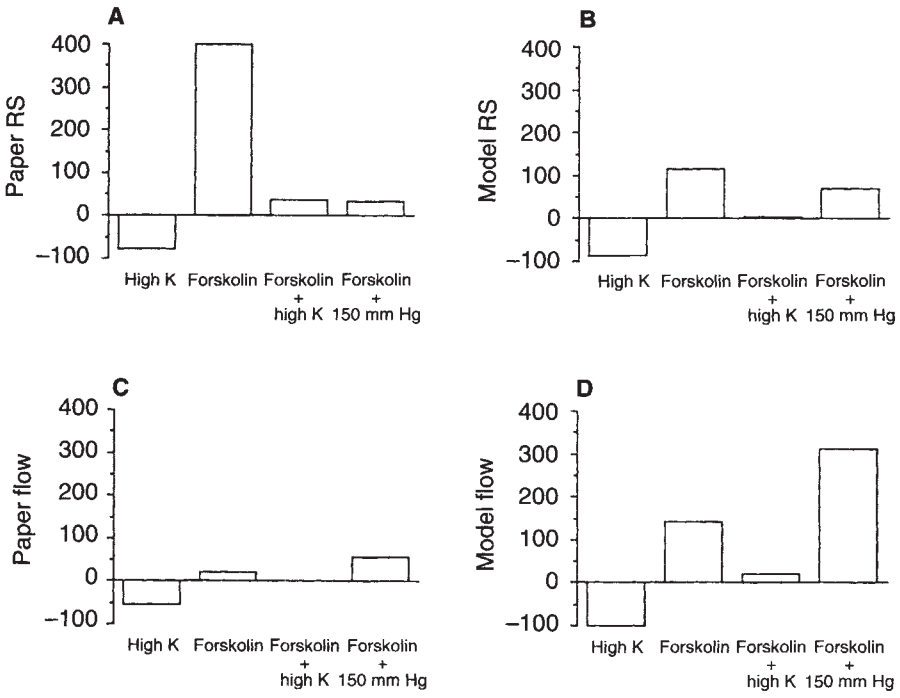

Fig. 4. *Comparison between renin secretion (RS) and renal blood flow (Flow) data as derived from experimental [51] and theoretical [74] studies.* All values represent % change from control. Left panels were derived from published experimental studies (**A** and **C**) whereas the right panels were derived from model calculations (**B** and **D**). All experimental studies were conducted in the isolated perfused kidney of the rat [52].

force will not be opposed by an increase in distending tension (as there is no increase in perfusion pressure) so the arteriolar radius will stabilize at a lower value and both RS and flow will be reduced. In contrast, the high pressure will increase both Ca_i and, therefore, the constricting force as well as the distending force with the result that the arteriolar radius will stabilize at a slightly higher value, decreasing RS but increasing flow.

The close qualitative match between experimental data and theoretical prediction lends support to the proposition that the model may reflect, albeit simply, the mechanisms determining flow and RS *in situ*. Of particular importance within the model are the central roles for physical equilibrium, myogenic autoregulatory behavior, and Ca in determining both flow and RS from the kidney. It would appear that these latter two phenomena cannot be considered independently *in situ* and that the secretory activity of the JG cells is set to a large extent by the myogenic determination of the hemodynamic environment to which the JG cells are exposed and to which they respond.

The model does not account for two major aspects of RS: firstly, the secretion of prorenin by the kidney, and secondly, the macula densa control of RS. There is strong evidence that these two phenomena are related [76–78]. The next section, therefore, attempts qualitatively to relate the macula densa control of proRS to the model described above.

Intrarenal control of prorenin secretion

Although it is well established that inactive (in terms of ability to generate angiotensin I from angiotensinogen in plasma) forms of renin are released by the kidneys of a number of species under a variety of conditions, and that the identity of circulating inactive renin is most likely prorenin, much less is known about the control of inactive compared to active RS. The main reasons for this discrepancy are methodical differences/difficulties, extrarenal sources of prorenin, and species differences.

Methodological differences/difficulties. The most widely used methods for assaying pro-forms of renin rely on measuring the ability of samples to generate angiotensin following activation of the inactive enzyme. Prorenin is then the difference between activated (total renin) and non-activated (active renin) samples. Methods for activation include acid dialysis [79], cryoactivation [80], and trypsin hydrolysis [81]. Although these methods are often designed to maximize renin activation within a particular laboratory and for a particular species, it can only be assumed that all the prorenin present is activated and renin destruction is minimized. Methodological differences in activation protocols also hamper comparison between species and laboratories. However, such indirect measurements of renin do have the particular advantage of estimating the contribution and potential contribution of the different renin molecules to the *in vivo* expression of RAAS. More recently, direct radiometric assays for renin and prorenin have been developed utilizing antibodies directed against the active site [82, 83] for active renin and against the pro-segment [84, 85] for prorenin. While these offer a quicker, simpler, and more direct estimate of (pro)renin (renin + prorenin), the measurements are of renin mass, not of biological activity. Neither direct nor indirect methods provide the complete answer, and it would seem that Slater and Haber's 1979 assessment of our view of prorenin as being "through a glass darkly" is still fairly appropriate [86].

Extrarenal sources of prorenin. Study of renal proRS in various species is complicated by possible interference from extrarenal sources of prorenin. Prorenin is demonstrable in the plasma of anephric man, but not in anephric sheep (D. Lush, unpublished observations), dogs [87] or rabbits. There have been conflicting reports regarding the presence of plasma prorenin following nephrectomy in rats, possibly due to methodological differences [88], although the submandibular gland has been implicated [89]. Prorenin is also associated with the

reproductive organs [90, 91]. Neither systemic nor local roles for extrarenal prorenin have been established, although it has been noted that prorenin tends to be associated with organs with high blood flow rates, which prompted the proposition that prorenin causes renal vasodilation [92]. Even if a role for extrarenal prorenin is found, the question still remains as to the relevance of extrarenal renin to the renally-mediated expression of RAAS. *In vitro* preparations provide the opportunity of studying renal prorenin secretion in isolation, yet few studies have been published compared to those in whole animals. Attempts at determining renal prorenin secretion in intact animals by measuring arteriovenous differences can be confounded by large coefficients of variation and the possibility of the renal clearance of prorenin as well as secretion [93].

Species differences. In most investigations of prorenin the measured variable is plasma prorenin. Given the variable contribution of extrarenal sources to circulating prorenin, as well as technical difficulties regarding plasma prorenin estimation [94], it is hardly surprising that no consistent picture emerges regarding possible variation in plasma prorenin between the commonly studied mammalian species (mouse, rat, rabbit, cat, dog, hog, sheep, human). However, in order to examine inter-species variation in the differential secretion of renin and prorenin by the kidney, *in vitro* preparations such as perfused kidneys and kidney slices offer two distinct advantages. First, renin and prorenin measured is of renal origin only. Secondly, the interfering effects of plasma are eliminated. Thus, *in vitro* preparations offer a potential means of studying species differences.

The most easily quantifiable difference between species is body size, and allometric analysis can be used as a comparative description of the effect of body size on biological parameters. This form of analysis can demonstrate functional trends and constraints not otherwise apparent from consideration of single species [95]. The relationship between the estimated percent of total renin released in the inactive, pro-form and body weight is illustrated in Figure 5 for eleven *in vitro* studies covering five species [76–78, 96 103]. The relationship is significant ($P < 0.02$), such that kidneys from animals of greater body weight tend to secrete a greater proportion of their renin in the pro-form. The possible significance of this observation will be discussed below with reference to the allometry of other aspects of renal function.

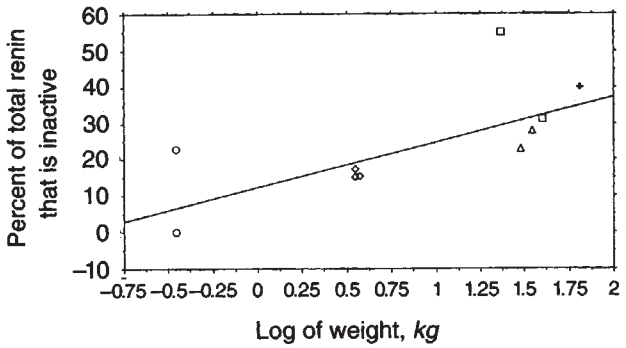

Fig. 5. *Direct relationship between the percent of total renin released in the inactive (prorenin) form and body weight as developed by allometric analysis.* Data were derived from eleven *in vitro* studies in the five species shown in the top panel. The relationship is significant ($P < 0.02$), such that kidneys from animals of greater body weight secrete a larger proportion of their total renin secretory package in the pro-form.

Allometric analysis of renal/cardiovascular function and its implication for (pro)renin secretion

Although methodological differences, extrarenal sources, and species differences are important, perhaps the major barrier to a fuller understanding of secreted prorenin is conceptual. Whereas the physiological role of active renin is to catalyze the production of angiotensin I from angiotensinogen and thereby determine the expression of RAAS, it has been difficult to ascribe a demonstrable role to prorenin within this hormonal system [93, 104], outside of its precursor function. The most obvious role for prorenin would be a contribution to the normal expression of RAAS through post-secretory activation—a role that follows naturally from the renin activating and angiotensin generating steps of the prorenin assays. From a variety of attempts to demonstrate post-secretory activation [105–108], the general consensus is that post-secretory activation of prorenin does not make a major contribution to the normal functioning of RAAS [93, 104]. Indeed, the most recent physiological role attributed to prorenin is that of a vasodilator [92]. In a radical and imaginative paper, it was speculated that prorenin binds to receptors in certain vascular beds where it undergoes a conformational change leading to activation and production of local angiotensin [92]. Yet in the vast majority of renin disorders, including diabetes mellitus with many of its complications characterized by excess prorenin [26], neither excess angiotensin II, its metabolites, nor abnormal renal vasodilation has been observed. What then must be the role of prorenin? There is one consistent assumption within the whole spectrum of views concerning the physiological role of circulating prorenin, even those that suggest a vasodilator role [92], which is that the contribution prorenin makes to the overall expression of RAAS is post-secretory and arises from an increase in its catalytic activity. Given the lack of clear evidence for this, and assuming that circulating prorenin of renal origin does fulfill a significant physiological role (in being the only precursor of renin) rather than represent a by-product of a default renin secretory pathway, the possibility remains highly likely that its physiological significance resides in its very lack of catalytic activity. The suggestion that differential secretion of renin and prorenin represents a true locus for controlling the expression of RAAS has been argued strongly by Noble [109].

Clues as to why the JG cell should adopt the curious tactic of determining the expression of RAAS (via angiotensin production) by controlling the overall activity as well as the amount of the renin it secretes may come from a consideration of the differing roles of the afferent arteriole and macula densa in the overall functioning of RAAS at the single nephron level. Recently, the implications for the whole organism of examining the functioning of RAAS from the perspective of the single nephron have been explored [110]. The major link between the whole organism and renal/nephron function is body size. Body size determines the basal metabolic rate, and renal function supports this metabolism.

It is possible, therefore, that the variation in prorenin secretion with body size demonstrated in Figure 5 is linked to other

size-related aspects of nephron function. The basis for such a link is considered below using data from the comprehensive analysis of the scaling of renal function in mammals by Calder and Braun [95]. The usual form of the allometric equation is:

$$Y = aM^b$$

where Y is the variable under study, M is body mass, and a and b are constants. Of particular interest is the exponent, b, which has a value close to 0.75 for mammalian basal metabolic rate. As expected, the gross renal support of mammalian metabolism scales to body size with similar values for b. Thus, RBF, GFR and urine production rate scale as $M^{0.77}$, $M^{0.72}$, and $M^{0.75}$, respectively. However, in terms of kidney structure, this equivalence does not hold. In particular, the number of glomeruli scales as $M^{0.62}$. Since the rate of increase in the number of glomeruli is less than the rate of increase in GFR, each glomerulus must handle a larger fraction of the GFR as body size increases. For example, from the data presented by Calder and Braun [95], the SNGFR of a 100 kg animal is twice that of a 0.1 kg animal, with the increase in SNGFR presumably accounted for by the increase in glomerular surface area. Similarly, as body size increases, each nephron must contribute more to the urine produced (that is, it must effectively reabsorb less).

Nephron function, through sodium/water reabsorption, represents the long-term efferent arm of RAAS. Thus, as body size increases and the contribution of each nephron to the normal excreted load also increases, it may be argued that a smaller induction of RAAS would be necessary to achieve normal salt/water balance. This implies that the JG cell needs to receive information concerning the size-related excretory load of the nephron. The macula densa mechanism may serve this function. Thus, a larger nephron from a larger animal may reduce activation of RAAS through the macula densa mechanism. In terms of a mechanism, NaCl concentration at the macula densa is regarded as a simple function of loop of Henle flow rate [111] and, presumably, SNGFR. In addition, there is an inverse relationship between macula densa Cl transport and RS [112]. In each of the nephrons of larger mammals, therefore, it is suggested that the higher SNGFR and tubular flow may increase Cl transport at the macula densa and inhibit RS. Thus, RAAS would be modulated downward, and the nephron would achieve its aim of a greater contribution to urinary excretion. Figure 5 suggests that this downward modulation of RAAS in larger animals is achieved by increasing the relative percentage of renin released in the pro-form rather than by decreasing the total amount of renin released.

Although the macula densa control of prorenin may occur through the intracellular pathway considered previously [4], the variation in the relative amounts with body weight suggested by Figure 5 may be viewed from an allometric perspective. Thus, theoretical modeling of the renal afferent arteriole suggests that the renin secretory response is exquisitely sensitive to disturbances of the physical equilibrium of the vessel and this, in turn, is influenced primarily by the myogenic autoregulatory response of the vascular smooth muscle. However, whereas the efferent arm of RAAS is sensitive to body size (through increases in SNGFR and single nephron urine flow with increased body size), it is likely that the afferent arm represented by the hemodynamic environment of the afferent arteriole is not. Renin release is inversely related to stretch-induced Ca influx, and stretch is influenced by blood pressure and afferent arteriolar radius [63]. Blood pressure is effectively size-independent [95] and measures of afferent arteriolar lumen diameter are similar in rabbits [65], dogs [74, 113], and humans [114]. This implies that, as body size increases, there is an increasing disparity between the magnitude of the renin-secretory activity of the afferent arm (through size-independent hemodynamic stimuli) and the ideal effect of this response on the efferent arm (through size-dependent tubular function). Thus, in a larger animal the same decrement in, say, blood pressure, may not require the same magnitude of renin secretory/angiotensin-mediated reabsorptive responses, since that animal's normal renal function is achieved with larger nephrons which excrete more of their filtered load. It is proposed that the macula densa, by sensing this aspect of tubular function, modulates the basal, baroreceptor-induced renin secretory response by varying the proportion of renin released in the inactive, pro-form. Thus, although the renin-secretory response of the baroreceptor is essentially size-independent, the proportion of renin released in the active form is varied by the macula densa to suit the size-dependent characteristics of the nephron.

Apart from the direct relationship between body weight and the percent of total renin released by the *in vitro* kidney in the inactive form (Fig. 5), there are a number of experimental observations which lend support to the proposal. First, differential release of (pro)renin is most often seen with maneuvers which alter electrolyte balance and tubular function. Noble and co-workers have shown that hemorrhage in the anesthetized rabbit [115] and in conscious and anesthetized sheep [116], calcium channel blockade in conscious sheep [117], and isoproterenol in anesthetized rabbits [78]—but not conscious sheep [118]—increase both forms of renin in parallel. On the other hand, dietary Na depletion in rabbits [119], and furosemide-induced diuresis in anesthetized rabbits [120] and sheep [121] is accompanied by a stimulation of renin and a reduction in prorenin. Morgan and co-workers [122, 123] investigated the influence of salt on the two forms at the level of the individual JG apparatus in rats. Dietary Na depletion was associated with an increase in the renin content of JG apparatuses with 30% of the renin in a pro-form, and a disappearance of prorenin from the circulation. Conversely, dietary Na loading was associated with a fall in JG renin content; no prorenin could be demonstrated in the JG apparatus, although 40% of plasma renin was in the pro-form [122]. These results are consistent with increased secretion of prorenin—with concomitant depletion of JG stores—with sodium loading and, presumably, increased delivery of NaCl to the macula densa. Indeed, increased microperfusion of the late proximal tubule in rats is associated with similar changes in JG renin and prorenin [123].

Secondly, there should be an increase in the proportion of circulating prorenin during infancy and adolescence secondary to increasing body size. Humans are born with a full complement of nephrons [124]. The rise in GFR which occurs during infancy and adolescence [125] is achieved by an increase in SNGFR secondary to increased glomerular capillary surface area [126]. From the above proposal, the rise in SNGFR

associated with maturation and increased body size may increase NaCl delivery to the macula densa and increase the relative proportion of renin released in the pro-form. The overall activation of RAAS would be blunted, thereby allowing each larger nephron to contribute more to overall urinary excretion. Fractional Na reabsorption is, indeed, less in the distal segments of older rats compared to younger rats, whereas Na delivery to the early distal tubule is greater [127]. Thus, the reduced distal Na reabsorption in older animals may be a consequence of reduced activity of RAAS mediated by increased NaCl delivery at the macula densa.

In humans, a comparison of infants under one year with children aged two to nine years suggested a reduced proportion of plasma prorenin in the older children [128]. Another study failed to demonstrate evidence for any change in the proportion of plasma renin in the pro-form with age [129]. A third demonstrated a greater proportion of total plasma renin concentration—but not activity (as measured by cryoactivation)—in the pro-form in adults compared to children (66% vs. 50%), but no statistical comparison was made [130]. A fourth study found few significant changes between a number of age groups [131] with a general trend towards increased proportions of prorenin was apparent from one month to 16 years, but the data were not subjected to regression analysis [131]. Nevertheless, two other studies with a total of 161 subjects have indicated highly significant negative correlations of plasma active renin with age and highly significant positive correlations of plasma prorenin with age, providing strong evidence that the proportion of plasma renin in the inactive form rises with age and, therefore, body size [132, 133].

Thirdly, the association with GFR provides additional support. Since it is proposed that the increased SNGFR normally associated with larger nephrons from large animals increases NaCl delivery at the macula densa and increases the proportion of prorenin secreted, there should be a direct relationship between prorenin secretion and (SN)GFR. It might be expected, therefore, that experimentally induced changes in GFR may also affect prorenin secretion. One particular study documents the relationship between prorenin and GFR [87]. Over a 12 hour period following the release of 48-hour ureteral ligation in dogs, GFR was positively and significantly correlated with plasma prorenin. In the context of the above proposal, the variation in prorenin is a consequence of the variation in (SN)GFR. Ablation of renal tissue reduces the number of nephrons available to support body metabolism. This produces compensatory renal growth in which GFR may return to normal [134]. From the above hypothesis, this could be associated with an increase in the relative secretion of prorenin from the remaining renal tissue, since compensatory growth is associated with an increase in nephron size, not number. Unfortunately, there are no studies of the effect of compensatory renal growth on the release of (pro)renin. SNGFR increases in both renal ablation and streptozotocin-induced diabetes in rats, apparently accompanied by increased NaCl transport at the macula densa [135]. This form of experimental diabetes is associated with reduced plasma active renin and increased plasma prorenin [136], lending support for the profound control of the macula densa on differential secretion of renin and prorenin, especially in diabetes.

Subcellular renin profiling and potential sites of impairment in diabetes mellitus and other cases of LRS

Although it is widely believed that the site of dysregulation in renin hyporesponsive diabetes and other LRS cases is intrarenal, only recently has enough evidence accumulated for critical evaluation. This section presents a number of possibilities and provides an assessment of the most promising for further research, particularly in diabetes where a fair amount of evidence is now available.

Several studies have attempted to identify the locus of renin impairment in diabetes mellitus, particularly those cases with low-renin diabetic hypertension [reviewed in 22]. Increased Na retention (presumably at the macula densa) was suggested as an explanation [137], but this remains controversial because several workers have argued that it is Cl [138] or perhaps osmolality [139] that triggers macula densa control of RS, not Na. As discussed above, this proposal may be enriched by arguing in terms of allometric analyses, but a detailed mechanism has not been advanced for diabetes. Decreased afferent arteriolar distensibility has also been suggested as an explanation [140] since distensibility affects RS [141]. However, the magnitude of the effect of decreased distensibility is small compared to other factors [142] and may be insufficient to explain the powerful suppression seen in diabetes. This is not to underestimate the significant contribution of the hemodynamic environment of the JG cell, but more to suggest that vascular distensibility can only make a limited contribution as predicted from equilibrium theory [63]. Furthermore, the renin hyporesponsiveness is also observed in cases without reported alteration of afferent arteriolar distensibility [13]. Lack of insulin was also suggested as an explanation for renal renin impairment [143, 144], and it was suggests that insulin is necessary for the normal renin secretory response [144]. This interpretation is at odds with studies showing excessive (or normal) RS in diabetes in the absence of insulin [140, 145, 146]. Furthermore, Cohen, Laurens and Fray [147] have shown an inhibitory effect of insulin on RS, which raises serious difficulties for the "insulin-dependent" theory [143, 144]. Deceased sympathetic nervous system activity has also been postulated as the site of impairment [3, 148], an effect observed in isolated perfused kidneys [19]. In the majority of studies, however, where renin secretory impairment has been observed, the sympathetic nervous system was normal [137, 149, 150] or could be activated [3], suggesting that depressed β-adrenergic responsiveness may be one manifestation of the pathogenesis of diabetes but upstream from a more fundamental impairment. Finally, body fluid volume expansion was suggested as the signal [3, 151]. Indeed, volume expansion has been observed [3, 7, 150], but normal blood volume has also been observed [137, 149]. Thus, the literature provides no satisfactory signal which accounts for the majority of the data on diabetes and low-renin diabetic hypertension at the extrarenal level.

An intrarenal locus for renin secretory impairment has been postulated. Christlieb [3] was the first to suggest that the defect was in renin production and storage (or content). He supported the proposition by showing a decreased renal renin content in alloxan diabetes [3, 150], and others have confirmed these findings in streptozotocin-induced diabetes [143]. However, these results may have to be reinterpreted because the renin

profile in streptozotocin-induced diabetes is biphasic: increasing during the first week of streptozotocin and decreasing by the fourth week (Fig. 1C). More recent studies have shown a decisive increase in renal renin content with streptozotocin [152]. Cohen, McCarthy and Rossetti [19] have shown a striking increase in renal renin content in rodents (BB/W) with spontaneous diabetes. Chronic stimulation caused a further twofold increase in renal renin content without significantly altering the secretory hyporesponsiveness in these rats [19]. As will be shown below, decreased renal renin content may not be a general phenomenon in renin disorders such as diabetes. Furthermore, the observation that diabetic rats with excess renin were still hyporesponsive to secretagogue [19], suggests that the impairment may be at a step distal to storage. Kidneys of spontaneously diabetic rats released half as much renin though they stored twice as much compared to non-diabetics [19]. The currently available evidence, therefore, suggests that the site of the impairment must be somewhere along the secretory cascade.

Subcellular fractionation, purification, and characterization of the major components of the renin secretory pathways have revealed significant clues as to the sites of impairment. Rough ER and Golgi transport vesicles, mature secretory granules, and plasma membrane subfractions with light microsomes have been identified as important components of the renin secretory cascade [4]. Subfractionation and purification studies have allowed further assessment of these components in the context of renin storage in a few animal models and in human diabetic hypertension in an attempt to gain further insights into the mechanisms of renin hyporesponsivity in LRS, especially as it relates to storage and processing.

It has been suggested that the processing of prorenin to renin is less efficient in diabetes and that this inefficiency may contribute to the impairment in patients with diabetes, hypertension or other renin hyporesponsivity syndromes [153], and an outline of possible hypotheses for the impairment has been suggested [154]. The abnormality may occur during (or shortly after) translation since several signals have been suggested to act through identifiable promoter elements upstream on the 12.5 Kb renin gene [155, 156]. This hypothesis predicts a decreased storage, an observation reported for only a small number of LRS cases [4]. Impairment shortly after translation might be expected to be revealed in the signal sequence and prosegment of the precursor molecule. This suggestion may be challenged by the observation that processing of the preprorenin molecule is independent of the signal sequence and further processing of the prorenin molecule is unaffected by the prosegment [156]. Furthermore, it was suggested that after the cleavage of the signal sequence in the ER and upon N-glycosylation in the Golgi the prorenin molecule is targeted to secretory granules for storage and release by regulative degranulation by an unknown mechanism [154]. It was also proposed that the unprocessed prorenin molecule emerging from trans-Golgi budding is released directly by constitutive vesiculation of immature Golgi transport vesicles [23], although a mechanism for the diversion to this default pathway has not been proposed. The constitutive vesiculation pathway was suggested to be prevalent in hypertension-induced renal disease [23]. Thus, sites of production and targeting may play a role in LRS, but equally significant are sites of processing and secretion.

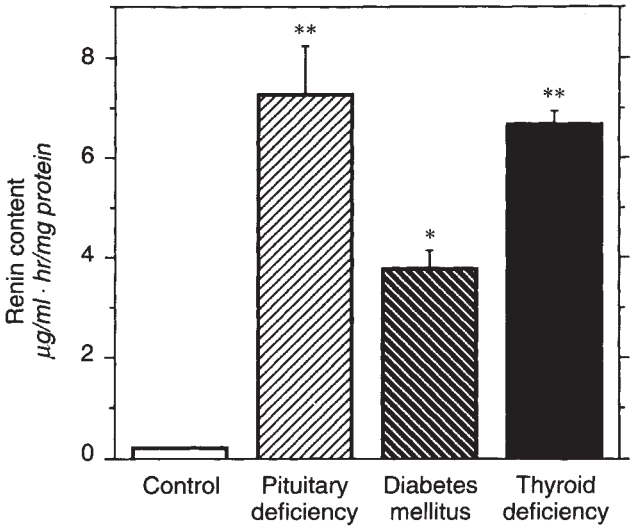

Fig. 6. *Renin content from partially purified subfractions of juxtaglomerular cells from three rodent models of low renin syndrome (LRS).* All LRS kidneys were removed from rats with low plasma renin activity and renin secretory hyporesponsiveness to acute stimulation. All values are mean ± SEM of renin specific activities after subfractionation (*$P < 0.05$; *$P < 0.01$) [24].

Several additional mechanisms in terms of processing and secretion may be proposed to explain the low PRA, high plasma prorenin activity, and renin secretory hyporesponsivity in diabetes and other models of LRS. The first is that JG cells from renin hyporesponsive diabetics and other cases of LRS have a higher Ca_i than normal. It is generally established that Ca_i is a final intracellular regulator of RS and that Ca_i is inversely related to RS [142, 157–161]. Consequently, it is customary to associate hypersecretion of renin with low Ca_i and hyposecretion with high Ca_i [162]. Since prorenin secretion has also been shown to be regulated by Ca_i, though not as thoroughly investigated as renin itself [163], it may not be unreasonable to expect that direct measurement of Ca_i in JG cells from renin hyporesponsive kidneys should have higher Ca_i than normal. Unfortunately, no direct measurements have been reported. Elevated Ca_i has been suggested to be characteristic of both low- and high-renin disorders [164]. Furthermore, hypertensive Blacks (generally believed to be low-renin) have a significantly lower Ca_i than hypertensive Whites (generally believed to be moderate-to-high-renin), at least in blood cells [165]. Therefore, a generalized elevated Ca_i in cells may not account for the renin hyporesponsivity.

The second proposal for the low PRA in renin hyporesponsive diabetics and other cases of LRS is secretory incompetence of stored protein rather than decreased storage. It is widely believed that decreased total renin content is responsible for the low PRA and secretory hyporesponsiveness [3]. In rodent models of diabetes mellitus renin content has been observed to be significantly elevated [19], thereby providing evidence against the renin depletion hypothesis. Figure 6 summarizes additional evidence for elevated renal renin content in diabetic rats and other cases of LRS where renal renin content is elevated substantially. Thus, although reduced renal renin content may account for the secretory impairment seen in

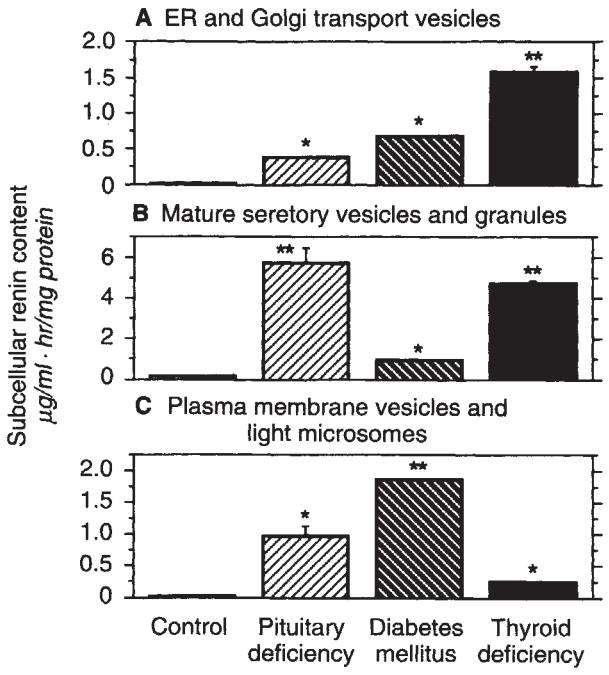

Fig. 7. *Subcellular particulate renin distribution in ER and Golgi transport vesicles (A), mature secretory granules (B), and plasma membrane vesicles and light microsomes (C) in there rodent models of low renin syndrome (LRS).* All LRS kidneys were removed from rats with low plasma renin activity and renin secretory hyporesponsiveness to acute stimulation. All values are mean ± SEM of renin specific activities after subfractionation (*$P < 0.05$; **$P < 0.01$) [24].

chronic salt loading [18] and mineralocorticoid excess [20], reduced content is not a generalized feature of LRS.

The third mechanism responsible for the low PRA and renin secretory hyporesponsivity is abnormal subcellular redistribution to divert the protein from the established regulative degranulation secretory pathway. Figure 7 summarizes a recent demonstration of subcellular renin profiles in pituitary deficiency (surgical), diabetes mellitus (congenital), and thyroid deficiency (surgical). ER and Golgi transport vesicles, mature secretory granules, and plasma membrane vesicles and light microsomes stored 13%, 72%, and 15%, respectively, of the particulate renin specific activity in JG cells from normal (control) rats. Whereas the 15% and 13% are substantially higher than what is expected from studies of other secretory proteins, the 72% in vesicles and granules is substantially lower than what is expected for either regulative degranulation [166] or constitutive vesiculation [167]. All three models of experimental LRS demonstrate significantly increased renin content in all subcellular compartments and an associated low rate of secretion, suggesting significant impairment in these models of renin disorder [4]. In pituitary deficiency the unusually large renin presence in transport vesicles and light microsomes associated with the plasma membrane subfraction suggests that this site may be a "sink" for renin destined for export through the regulative degranulation pathway [13]. In the granular subfraction, despite the 35-fold increase in renin specific activity over control, pituitary deficient rats secreted an amount indistinguishable from controls [11–13]. In congenital diabetes mellitus the transport vesicles and light microsomes in the plasma membrane subfraction appear to be the key sites of storage (Fig. 7). In thyroid deficiency, on the other hand, ER and Golgi transport vesicles and mature secretory granules appear to be the major sites of storage (Fig. 7). Thus, in all three cases of LRS, abnormal subcellular redistribution of renin away from the established regulative degranulation secretory pathway may account for at least some aspects of the secretory impairment.

In humans where there have been demonstrated both racial- and disease-related differences in PRA, recent studies have identified a subcellular basis of these differences [22]. Blacks, it is well recognized, are classified as "low-renin" because of their lower PRA and secretory hyporesponsivity [24]. Although JG cells from Blacks store considerable amounts of renin, the absolute levels are statistically significantly lower than Whites, and this may in part account for the differences in PRA [168]. In terms of total cellular renin, however, Blacks and Whites store similar amounts, but whereas Blacks store a major fraction of total as prorenin in mature secretory granules, Whites store their major fraction in ER and Golgi transport vesicles and heavy microsomes displaced from identifiable mature secretory granules [22]. In addition, Blacks store an unusually large proportion of total cellular renin in transport vesicles and light microsomes of the plasma membrane subfraction. Since constitutive vesiculation may also originate from this site [169], it may account in part for the excess prorenin secretion in Blacks [170]. A fair number of diabetic hypertensives, it has been suggested, may also be classified as renin impaired, and the cellular basis of the impairment was postulated to be reduced JG cell renin storage, without consideration of prorenin [3]. Elevated prorenin is a common observation in human diabetes [153] and one explanation for the excess secretion of prorenin may be the excess prorenin presence in JG cells. The major fraction of subcellular prorenin was localized to the ER and Golgi transport vesicles and heavy microsomes, suggesting a constitutive vesiculation secretory pathway similar to that proposed for prorenin in the renal ischemia [23].

The fourth proposal for the renin secretory impairment is dysfunction of chemiosmotic components in the secretory granule. It has been shown that renin secretory granules have a number of chemiosmotic components (molecules involved in coordinated chemiosmotic transport of ions and water) that are required for assistance to complete processing of prorenin to renin and for engagement to the initial swelling events of exocytosis [21]. The two shown to be most significant are a proton ATPase and a KCl-H exchanger translocator [21]. A proton ATPase was shown to be required to maintain the intragranular matrix at pH 5.56 ± 0.04 [21]. Acidic pH in the range of pH 5 to 6 may be required for maximal prorenin convertase(s) activity [171, 172]. Under normal conditions in isolated granules from animal models, it was observed that hypokalemia and/or hypochloremia were inhibitory to renin release. It was also observed that both hyperkalemia and hyperchloremia were stimulatory but the effect was sharply dependent on the acid-base status [21]. Between cytosolic pH 6 to 7 hyperkalemia and hyperchloremia were without effect, whereas at both acidic and alkalotic pHs renin release from granules was stimulated by hyperkalemia and hyperchloremia [21]. It was postulated that at acidic pHs the proton ATPase in the granular membrane is the primary chemiosmotic component

responsible for inward proton translocation to acidify the granular matrix and activate prorenin convertase(s) to process prorenin to renin. Impairment in the proton ATPase activity or cytosolic alkalinity would consequently prevent inward proton pumping and inactivation of the convertase(s) thereby yielding a decreased intragranular renin activity [21]. It was also shown that at alkaline pHs the KCl-H exchanger in the granular membrane is the primary chemiosmotic component responsible for granular swelling and exocytotic release of granular content [21]. Impairment in the KCl-H exchanger would consequently promote increased granular storage of renin and prorenin without secretion. Thus, the fourth proposal is that an impairment in the proton ATPase in the granular membrane leads to impaired prorenin processing and an impairment in the KCl-H exchanger leads to incompetence in RS. Several lines of evidence support this hypothesis [4]. Biochemical characterization of these molecules are now required.

The final mechanism which may partly explain the low PRA and secretory hyporesponsivity in some renin hyporesponsive disorders is impairment in export at the level of light microsomes and plasma membrane. A renin anchorage system has long been recognized in the plasma membrane of JG cells [13, 173, 174]. Renin is cleavage from the anchorage system by trypsin, melittin, kallikrein, phospholipase A_2, lysolecithin, and high salt. Ca and Ba increase cleavage, whereas Mg and Mn decrease it. Translocation is stimulated by calmodulin (in the absence of Ca) and KCl, and inhibited by La [4]. In models of renin disorder stimulation of secretion not infrequently leads to increased renin trapping in the transport vesicles and light microsomes of the plasma membrane subfraction and to decreased export to the extracellular space [13]. Furthermore, in animal models of diabetes the major fraction of cellular renin was observed in light microsomal fraction containing plasma membrane transport vesicles (Fig. 7), suggesting that a dysfunction in the plasma membrane and light microsomes subfraction of JG cell may play a significant role in the renin secretory hyporesponsivity.

Summary and outlook

It is generally accepted that RAAS plays an important role in (patho)physiology (physiology with imminent pathophysiology) of cardiovascular and renal regulation. The classical baroreceptor, neurogenic, hormonal, and macula densa mechanisms regulate renin expression at the cellular level by Ca, cAMP, and chemiosmotic forces. The baroreceptor mechanism operates through Ca_i as a second messenger which activates K and Cl channels in the surface membrane and deactivates a KCl-H exchange transporter in the secretory granular membrane. The neurogenic mechanism, partly through modulating Ca_i, but mostly by stimulating the generation of cAMP as a second messenger, plays an important role both in enhancing transcription and in stimulating RS. Hormones and neurohormones affect either Ca or cAMP pathways. The macula densa mechanism involves the processing of prorenin to renin for subsequent secretion by a chemiosmotic mechanism. This role of the macula densa and its unique anatomical juxtaposition with the juxtaglomerular cell may explain the diversity of plasma prorenin presence in mammals. Allometric analysis shows that animal size correlates directly with plasma prorenin levels, and those levels of prorenin correlate positively with GFR and nephron size, and chemiosmotic flux at the macula densa. The macula densa therefore plays an important role in differential release of renin and prorenin in response to physiological challenges and pathophysiological conditions by virtue of its control of chemiosmotic flux.

Analysis of a few clinical disorders suggest one striking similarity: suppressed RS. In an attempt to observe further the (patho)physiological mechanisms of this secretory hyporesponsivity, a broader picture of several renin disorders have emerged which when probed at the subcellular level reveals a multiplicity of impairment sites; the following is a representative example of the foregoing discussion. First, low PRA signifies an impairment in renin production and secretion which results from abnormal JG cell secretory expression and not from extrarenal factors. Second, although there may be a slight impairment in signal transduction at the plasma membrane in diabetes mellitus with neuropathy and perhaps in some cases of hypertension, the more pronounced impairment(s) is at the intracellular level downstream from the generation of Ca_i as an intracellular messenger.

Third, reduced cellular renin content is not entirely responsible for the low PRA in all cases of LRS because in both congenital diabetes in rodents and in human non-insulin-dependent diabetic hypertension as well as in other cases of LRS, such as pituitary deficiency and thyroid insufficiency, renal renin content is significantly elevated. It should be noted, however, that reduced renin production (and therefore subnormal storage) may be significant in cases such as primary aldosteronism and chronic salt excess, where suppression may be at the level of translation and transcription.

Fourth, impairments may be localized at various intracellular sites along the secretory pathway—ER and Golgi transport vesicles, mature secretory granules, and plasma membrane—as evidenced by subcellular fractionation studies. Reduced trafficking from ER and Golgi network to mature secretory granules may be a feature of cases such as diabetes and thyroid hypofunction, where renin storage appears high in these compartments and where the primary mode of secretion appears to be constitutive vesiculation of prorenin. Reduced release from mature secretory granules may be a feature of cases such as thyroid insufficiency and pituitary deficiency where granular content is in excess of normal but secretion is impaired.

Fifth, prorenin is the predominant species secreted in most cases of LRS, including diabetes and diabetic hypertension. The unusually large fraction of prorenin demonstrated at various sites along the secretory pathway suggests that incomplete processing of the precursor molecule may be one reason for the lower active renin appearing in plasma.

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